Intravenous SNF472 (a novel inhibitor of vascular calcification) does not affect bone histology and histomorphometry in healthy and uremic animal models

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Introduction to SNF472

- i.v. formulation of **IP6**: myo-inositol hexaphosphate
- **IP6** in blood (< 0.3 µM) and in cells (10-100 µM)
- Potent modulator of calcification
- **SNF472** Expected **therapeutic** concentrations 2-3 µM
- **SNF472** in **Phase 2** clinical development for cardiovascular calcification in ESRD dialysis patients and calciphylaxis

**Physico-chemical MoA**: SNF472 prevents cardiovascular calcification by blocking Ca-crystal formation/growth
SNF472 has shown efficacy in the prevention and progression of CV calcification induced by vitamin D and in the prevention of calcification in uremic models.

Prevention of CV calcification in a vitamin model. SNF472 administered by 4h i.v. infusion. Data presented at ERA-EDTA Meeting, Vienna 2016.


Inhibition of progression of CV calcification in a vitamin D model. SNF472 administered s.c. Manuscript in preparation.
SNF472: Therapeutic margin

[Ca] in blood = 2200-2700 μM

- **Bolus/Side effects**: ↓Ca
- **In vivo efficacy (EC50)**
- **In vitro efficacy**

**Bolus**
- SNF472: Therapeutic margin
- [SNF472] (µM) = 450

**Infusion**
- [SNF472] (µM) = 150

- **CHELATION**
  - Bolus
  - Infusion

- **EFFICACY**
  - [SNF472] (ng/ml):
    - 3·10\(^5\)
    - 10\(^5\)
    - 3·10\(^4\)
    - 10\(^4\)
    - 3·10\(^3\)
    - 10\(^3\)
    - 3·10\(^2\)
SNF472: Therapeutic margin

- **In vitro efficacy**
  - Bolus/Side effects: ↓Ca

- **In vivo efficacy (EC50)**
  - [Ca] = 2200-2700 μM

**Efficacy**

- Start chelating free calcium in blood
- Selective and potent binding to HAP
- Stop HAP cristal growth

**Selective and potent binding to HAP**

- X 100

**Therapeutic margin**

- [SNF472] (ng/ml)
  - 3·10^2
  - 3·10^3
  - 3·10^4
  - 3·10^5

- [SNF472] (μM)
  - 0.45
  - 1.5
  - 4.5
  - 15
  - 45
  - 150
  - 450
Antecedents on bone (In vitro)

- IP6 does not impair the ability of osteoblasts to synthesize a collagenous matrix, express ALP or differentiate to produce specific bone matrix proteins\(^1\)

- Ti-IP6 surfaces obtained are not cytotoxic for MC3T3-E1 osteoblastic cells and significantly induce the gene expression of osteogenic markers, indicating the osteogenic potential of these surfaces\(^2\)

- IP6 inhibits osteoclastogenesis and bone resorption activity in mature osteoclasts but without affecting viability or inducing apoptosis\(^3\)

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\(^1\)Addison et al. Bone 2010;46:1100-1107
\(^2\)Córdoba et al. ACS Appl Mater Interfaces 2016;8:11326-35
\(^3\)Arriero et al. PLoS One 2012;7:e43187
Antecedents on bone (In vivo)

- Animals with an IP6-enriched diet had a reduced loss of BMD caused by estrogen deficiency in ovariectomized Wistar rats\(^1\)

- Subjects with a high dietary IP6 intake had higher values of BMD in the calcaneus, lumbar spine and femoral neck (Retrospective study, N=1473)\(^2\)

- Postmenopausal women: higher urinary levels of IP6 correlated with higher BMD in the lumbar spine and femoral neck (N=180)\(^3\)

- Postmenopausal women: higher physiological levels of IP6 are correlated with a lower bone mass loss during a period of 12 months (Prospective, N=157)\(^4\)

\(^1\)Grases et al. *J Med Food* 2010;13(6):1301-6
\(^3\)Lopez-Gonzalez et al. *Front Biosci* 2010;1,2;1093-1098
To study the effect of SNF472 administration in bone properties in vivo in healthy and uremic animals
AIM: to study the effect of SNF472 administration in bone properties in healthy dogs

- 0.9% Physiological saline
  - 15 min i.v. infusion
  - E.o.d. administration

- 25 mg/kg SNF472
  - 15 min i.v. infusion
  - E.o.d. administration

39 weeks

- Bone histomorphometry
- Histological analysis
9-month toxicology in dogs

Trabecular parameters

![Images of trabecular bone samples for different doses and genders.](image)

<table>
<thead>
<tr>
<th>Dose</th>
<th>Gender</th>
<th>Bone volume fraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mg/kg</td>
<td>Female</td>
<td>20%</td>
</tr>
<tr>
<td>0 mg/kg</td>
<td>Male</td>
<td>20%</td>
</tr>
<tr>
<td>25 mg/kg</td>
<td>Female</td>
<td>20%</td>
</tr>
<tr>
<td>25 mg/kg</td>
<td>Male</td>
<td>20%</td>
</tr>
</tbody>
</table>
9-month toxicology in dogs

Trabecular parameters

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trabecular thickness (mm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Trabecular separation (mm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Trabecular number (mm⁻¹)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
9-month toxicology in dogs

Cortical parameters

- 0 mg/kg dose
- 25 mg/kg dose

Cortical area fraction (%): 0,0, 0,5, 1,0, 1,5

Cortical thickness (mm): 0,0, 0,5, 1,0, 1,5

Images show cortical area fraction and thickness for different doses and genders.
9-month toxicology in dogs

TRAP staining

Cortical bone

[A] Control Female  [B] Control male

[C] SNF472 Female  [D] SNF472 male

Trabecular bone

[A] Control Female  [B] Control male

[C, F] Rat femoral positive controls
9-month toxicology in dogs

Von Kossa staining

[A] Control Female  [B] Control male
[C] SNF472 Female  [D] SNF472 male
[E, F] Rat femoral positive controls
Preliminary study in uremic rats

- **AIM:** to study the effect of SNF472 administration in bone properties in uremic rats

<table>
<thead>
<tr>
<th>Condition</th>
<th>Treatment</th>
<th>Group Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>i.v. saline 0.9%</td>
<td>N=6</td>
</tr>
<tr>
<td>Nx 5/6</td>
<td>i.v. saline 0.9%</td>
<td>N=10</td>
</tr>
<tr>
<td></td>
<td>SNF472 5 mg/kg</td>
<td>N=11</td>
</tr>
</tbody>
</table>

- **Crl:** OFA SD
- **Calcification**
- **Bone histomorphometry**
- **Mechanical properties**

8 weeks
Preliminary study in uremic rats

**Aorta calcification**

Statistical analysis: One-way ANOVA. (*) difference vs sham, (#) difference vs vehicle, p < 0.05.
Preliminary study in uremic rats

Bone architecture

Sham

Calcified uremic

Non-calcified uremic

SNF472
Preliminary study in uremic rats

### Mechanical properties

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Non-calcif</th>
<th>Calcif</th>
<th>SNF472</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maximum Force (N)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>135.12</td>
<td>96.88</td>
<td>79.16</td>
<td>108.93</td>
</tr>
<tr>
<td><strong>Young's modulus (MPa)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>21697.92</td>
<td>43557.74</td>
<td>4319.40</td>
<td>49711.87</td>
</tr>
<tr>
<td><strong>Breaking stress (MPa)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>267.32</td>
<td>270.86</td>
<td>51.42</td>
<td>384.62</td>
</tr>
<tr>
<td><strong>Breaking strain (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2.46</td>
<td>1.81</td>
<td>3.04</td>
<td>1.47</td>
</tr>
</tbody>
</table>
Conclusions

1. Femurs from SNF472 treated dogs are physiological with an organized and connected trabecular network and regular cortical bone.

2. Bone resorption by osteoclasts is not enhanced in dogs by SNF472.

3. SNF472 treatment in dogs does not affect bone mineralized tissue fraction.

4. Calcified uremic rats present huge porous tissue at the epiphysis level and a very thin cortical bone with porous bone occupying the diaphysis.

5. Femurs from calcified rats are breakable because of the thin cortical bone, while femurs of non-calcified rats are stiffer and more resistant.

6. Femurs from SNF472-treated rats do not differ from femurs from sham and non-calcified Nx5/6 rats.
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